Biometra
An Analytik Jena Company

TGGE MAXI System

Instruction Manual

Ver. 05/12

Model	**Order No.**
TGGE MAXI System	**024-200**
TGGE MAXI System (international)	**024-290**

Please read these instructions carefully before using this apparatus!

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This document describes the state at the time of publishing. It needs not necessarily agree with future versions.

Subject to change!

Contents

1. Introduction

Temperature Gradient Gel Electrophoresis is a powerful technique for the separation of nucleic acids or proteins. The TGGE method, which is covered by patents, uses the temperature dependent changes of conformation for separating molecules (for review see Reference 1).

Since the introduction of the first commercial available TGGE apparatus in 1989, temperature gradient gel electrophoresis has gained high interest in scientific and clinical research laboratories due to the unprecedented resolution capability and easiness of analysis. The range of scientific publications using the TGGE method is broad and covers all disciplines which use molecular biology methods: e.g. Oncology 2-4, Virology 5,6, Immunology 7,8, RNA Viroid Research 9-12, Prion Research 13, Population Analysis 14-15. The TGGE method has also been used for quantitative analysis in industry 16-17 and for conformational analysis of proteins 18-19.

1.1 Principle of the method

Conventional protein or nucleic acid electrophoresis separates molecules according to their size or charge. **TGGE** adds a new parameter for separation, namely the **melting behavior of a molecule**. The melting behavior is determined by primary sequence and secondary and tertiary structure of the molecule and can be changed by external influences like temperature, salt concentration, pH etc.

During electrophoresis the sample migrates along a temperature gradient. As the temperature rises the molecules start to denature. Working with PCR fragments for example electrophoresis starts with double stranded molecules. At a certain temperature the DNA starts to melt, resulting in a fork-like structure (partial single strand, see Figure 1). In this conformation the migration is slowed down compared to a completely double stranded DNA fragment (of same size). Since the melting temperature strongly depends on the base sequence, DNA fragments of same size but different sequence can be separated. This is used in mutation detection where PCR fragments of identical size but different sequence are separated. Thus TGGE not only separates molecules but gives additional information about melting behavior and stability.

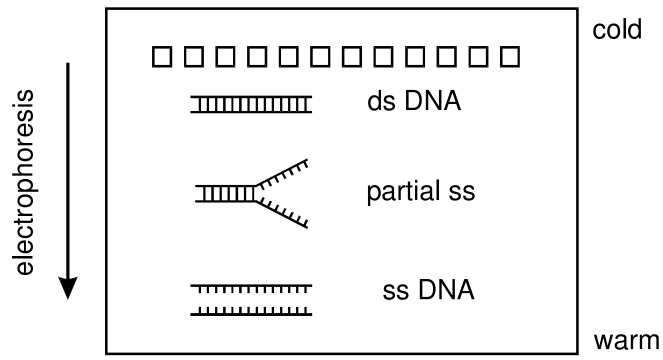


Figure 1: Different conformations of DNA during temperature gradient gel electrophoresis.

1.2 Field of Applications

The Biometra TGGE MAXI system separates molecules in a temperature gradient. Unlike chemical gradients the peltier driven temperature gradient is controlled by a microprocessor

and thus is providing unmatched reproducibility. The most common application for TGGE is mutation analysis of PCR fragments. The DNA molecules become separated in the temperature gradient their melting behavior. With TGGE PCR fragments that only have single base substitutions can be resolved. But, the TGGE system is not restricted to the analysis of DNA fragments only. It can be used for versatile applications like:

- Mutation analysis
- Heteroduplex analysis
- DNA methylation studies (Imprinting)
- Differentiation of amplicon and competitor for quantitative DNA analyses
- Fidelity assay for thermostable polymerases
- Secondary structure analysis of RNA
- Analysis of dsRNA molecules
- Thermal stability analysis of proteins
- Protein/protein or protein/ligand interaction analysis

1.3 Special features

1.3.1 Peltier powered linear temperature gradient

The heart of the TGGE MAXI system is the temperature block which is powered by peltier technology. Thanks to precise microprocessor control a linear temperature gradient is generated providing maximum reproducibility. Thus assay conditions can be much better controlled compared to conventional chemical gradients (DGGE) or temporal gradients using water baths.

The Biometra TGGE system is available in two formats. The standard TGGE “mini” system operates small gels and is therefore ideally suited for fast, serial experiments. The TGGE maxi system provides a large separation distance and allows high parallel sample throughput.

Using the Biometra TGGE system it is very easy to separate samples either parallel or perpendicular to a temperature gradient. All that has to be changed is the position of the buffer tanks. Whereas perpendicular TGGE is mainly used for the optimization of separation conditions, parallel TGGE allows fast analysis of multiple samples.

Perpendicular TGGE	Temperature gradient is **perpendicular** to the electrophoretic migration: One sample is separated over a broad temperature range to determine the optimum temperature gradient or to analyze temperature dependent changes in conformation
Parallel TGGE	Temperature gradient is **parallel** to electrophoretic migration: multiple samples are separated in parallel

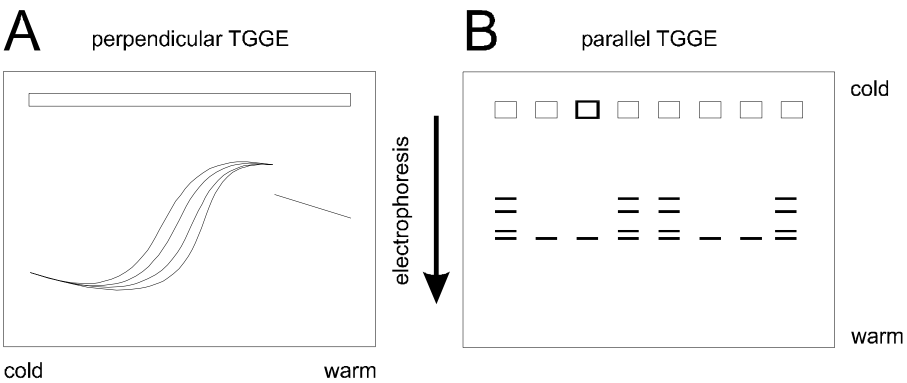


Figure 2: Typical results after perpendicular TGGE (A: temperature gradient from left to right) and parallel TGGE (B: temperature gradient from top to bottom).

1.3.2 Sensitivity

Because of the small amount of material used for separation, DNA or RNA fragments appear as fine bands which can be clearly distinguished from each other. Even complex band patterns can be analyzed due to the high resolution capability of the gradient block. Comparing the TGGE method with other screening methods like SSCP the superior performance of the TGGE method becomes evident.

In contrast to direct sequencing TGGE also detects mutations in mixed DNA samples. Whenever heterozygous DNA is to be analyzed, direct sequencing will not give a clear signal at the position of the mutation. This is especially the case if the mutated gene is masked by a high background of normal cells. TGGE reliably detects mutations in a 1:10 dilution (and higher) of wildtype DNA.

1.3.3 Patented technology

TGGE is protected by patents in most countries of the world. The patent for the TGGE method is held by Qiagen AG, Hilden. Biometra is the exclusive licensee for manufacturing and distribution of TGGE instrumentation.

1.4 Technical specifications

TGGE MAXI System	Electrophoresis unit with temperature gradient block and two removable buffer chambers, controller, power supply, starter kit.
TGGE MAXI Electrophoresis unit	
Temperature gradient formation	High performance Peltier technology
Block size	20 x 20 cm
Temperature range	5 – 80 °C
Linear temperature gradient	maximum 45 °C
Temperature accuracy	± 0.3 °C
Temperature uniformity	± 0.5 °C
Glass plate size	23.5 x 23.5 cm
Gel size	approx. 20 x 20 cm
Separation distance	parallel: 16 cm perpendicular: 19 cm
Dimensions (L x W x H)	42.3 x 42.3 x 33.3 cm
Weight	22 kg
TGGE MAXI System Controller	Microprocessor driven control of temperature gradient and electrophoretic parameters
Current	maximum 500 mA
Voltage	maximum 400V
Wattage	maximum 50W
Program stores	up to 100 programs can be stored
Program modes	constant Voltage V/h integration Programs can contain different steps (pre-run, pause, run)
Display	LCD display, 4 lines, English / German
Interfaces	parallel (Centronics), serial (RS 232)
Dimensions	31 x 22 x 11.5 cm
Weight	3.5 kg
Voltage	110 / 230 V
TGGE MAXI Power Supply	
Dimensions	29.5 x 22 x 8 cm
Weight	6.5 kg
Transformer	450 VA

1.5 Legal Notes

1.5.1 Copyright

Biometra is pointing out that applied company and brand names are usually protected trade marks.

The **TGGE** method is covered by patents issued to Diagen (now QIAGEN GmbH).

The polymerase chain reaction **(PCR)** process is covered by patents issued to Hoffman-La Roche.

Acryl-Glide™ is a trademark of Amresco Inc.

Biometra is a trademark of Biometra GmbH.

The **POLAND** software service established by Gerhard Steger, Department of Biophysics, University of Duesseldorf, is available by internet:

www.biophys.uni-duesseldorf.de/POLAND/poland.html

1.5.2 Liability

Biometra is not liable for damages and injuries caused by use not considering these operating instructions in parts or completely.

1.5.3 Meaning of the Instructions

Biometra recommends that you first read these instructions carefully. This operation instruction is part of the product and should be kept over the full life-time of the instrument. It should also be forwarded to subsequent owners and users. Make sure that additions and updates are inserted into the operation instructions.

2. Safety and Warning Notices

2.1 Definition of Symbols

Symbol	Definition
	Caution! Refer to instruction manual!
	Danger! High voltage!
	Fragile!
	Danger! Hot surface!

2.2 Safety instructions / general remarks

The thermoblock will reach high temperatures during operation. The thermoblock can burn you.

The TGGE system contains no user serviceable parts. Do not open the housing instrument. Service and repair may only be carried out by the Biometra Service department or otherwise qualified technical personal.

Do not use the instrument when damages of the housing, block, cable or other parts are visible.

Prior to connecting the unit to the power source please ensure that the voltage setting at the fuse holder at backside of the power supply is set to the required voltage.

Unplug the power cable before you open the TGGE system. Danger of electric shock!

The thermoblock is covered with Teflon film. Avoid damaging this film.

Make sure that the appliance connector and the plug of the supply cord are accessible, so you can separate the instrument from the mains.

Connect the TGGE system to a grounded socket.

Appropriate safety regulations must be observed when working with infectious, pathogenic or radioactive material. Ask the responsible local safety inspector for details.

Place the TGGE system on a stable, non flammable surface in a dry, safe environment.

Ensure that both the ventilation slits of the TGGE block not clogged by dust or other material. Danger of overheating!

Do not fill buffer chambers above marking for maximum level

Do not move instrument during operation

In case of strong condensation under the safety lid stop run, dry instrument and re-start

If buffer has been spilled on the electrophoresis unit, clean it carefully before start of electrophoresis

Do not use paraffin oil on the thermoblock.

Switch off power before removing the safety lid

This instrument is designed and certified to meet EN 61010-1 safety standards. It should not be modified or altered in any way. Alteration of this instrument will void the warranty, void the EN61010-1 certification, and create a potential safety hazard.

Do not use alcohol (e.g. methanol, ethanol), organic solvents or abrasives to clean the instrument.

3. Installation

3.1 Content of delivery

1) **Electrophoresis unit**
including thermoblock, buffer chambers, safety lid

2) **Controller**
control of electrophoresis parameters (Voltage) and temperature gradient

3) **Power supply**
power supply for electrophoresis unit and controller

4) **Starter Kit**
Glass plates, sealings, cover films, polybond films, buffer wicks, binder clips, applicator strip, sample Acryl Glide™* (*only in Germany)

5) **Manual**

Please keep the original packaging material for return shipment in case of servicing.

TGGE MAXI System, 230/115 V (Germany only) Electrophoresis unit with high precision gradient block, 2 buffer chambers for variable positioning, controller, power supply, manual, TGGE MAXI Starter kit 024-204	024-200
TGGE MAXI System, 230/115 V (International) Electrophoresis unit with high precision gradient block, 2 buffer chambers for variable positioning, controller, power supply, manual, TGGE MAXI Starter kit 024-294	024-290

3.2 Unpack and Check

Unpack and carefully examine the instrument. Report any damage to Biometra. Do not attempt to operate this device if physical damage is present.

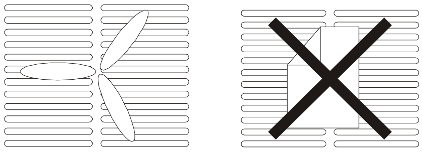
Please keep the original packing material for return shipment in case of service issues

!! Attention !!

Please fill out and send back the warranty registration card. This is important for you to claim full warranty.

3.3 Installation Conditions

- Place the TGGE system on a stable surface in a dry, safe environment.
- Let equilibrate the TGGE system to room temperature before starting operation.
- Make sure that the appliance connector and the plug of the supply cord are accessible, so you can separate the instrument from the mains.
- Connect the TGGE system to a grounded socket.



Ensure that the ventilation slits at the electrophoresis unit are unobstructed.
Insufficient ventilation can cause overheating of the instrument.

Prior to connecting the unit to the power source please ensure that the voltage selector at the back side of the power supply is set to the required voltage.

Danger of electric shock! Unplug the power cable before you open the TGGE system.

3.4 Operation Voltage

Important: Prior to connecting the TGGE system to the mains, make sure that the voltage setting of the power supply is in accordance with your mains voltage. The set operating voltage is displayed in the small window of the fuse holder at the backside of the power supply.

The TGGE system can operate at 110 or 230 Volt. To change operation voltage of the TGGE system, switch off the instrument and disconnect the mains plug. Press the two clips both ends of the fuse holder and excerpt it. Pull out the cream coloured insert from the fuse holder and turn it by 180°. Insert it again and control the voltage displayed in the small window of the fuse holder. If the setting is correct insert the fuse holder to the power supply again.

Backside power supply

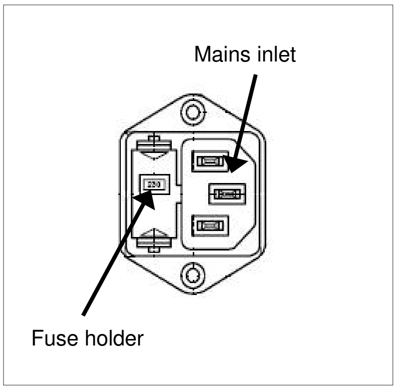


Figure 3. TGGE system fuse holder

3.5 Setup

1) Connect electrophoresis unit and controller.

2) Connect controller and power supply

3) Connect power cables from the safety lid to the controller

The buffer chambers can be placed in two orientations, depending on the direction of the temperature gradient. Be sure that the orientation is correct. The markings on the gradient block (L0 to L10) indicate the direction of the temperature gradient. The direction of electrophoresis (minus to plus for nucleic acids) is indicated on the safety lid.

4. Considerations for successful TGGE experiments

TGGE is a powerful technique to separate molecules of same size, but different sequence. Nevertheless, every DNA fragment has its own characteristics and three steps have to be taken before successful analysis of multiple samples in parallel TGGE can begin. Each of the following steps is described in detail in section.

Step 1 Check the sequence of your PCR fragment in Poland analysis. The Poland computer program (http://www.biophys.uni-duesseldorf.de/POLAND/poland.html) calculates the melting behavior of dsDNA molecules. Poland analysis can predict, whether a fragment is suited for TGGE or not. Analysis is available online and free of charge (see section 4.3.1).

Melting profile is ok
Poland analysis shows a satisfying profile.

Proceed with step 2

Melting profile is not ok
If Poland analysis shows that the fragment in its current state is not suited for TGGE, optimize your primer design. **Never** try to separate samples in TGGE if the calculated melting profile is not ok. **Back to step 1**

Step 2 If the Poland analysis shows a suitable melting profile you should test separation conditions in a perpendicular TGGE. In perpendicular TGGE, a large aliquot of the sample runs over a broad temperature range. The result of parallel TGGE allows identification of the temperature gradient for parallel analysis.

Perpendicular gel is ok
Perpendicular TGGE shows a nice melting curve.

Proceed with step 3

Perpendicular gel is not ok
If perpendicular analysis does not show the expected melting profile, check sequence again in Poland analysis. Also check purity of chemicals and electrophoretic conditions.
Do not try samples is parallel TGGE, as long as the perpendicular gel does not show a defined melting curve.
Back to step 2

Step 3 Set up a parallel gel with the temperature gradient derived from the perpendicular gel. Separation can be optimized by varying the temperature gradient and voltage.

4.1 Adaptation of protocols from the TGGE "mini" system

In contrast to the „mini" system, the TGGE maxi system works with gels of 1mm thickness. Therefore electrophoretic parameters have to be adapted. This can be done by running a perpendicular gel or by performing a time chase experiment. Both techniques are described in detail in section 4.4 and 4.5.

With the TGGE maxi system the settings for T1 and T2 have been omitted. The temperature gradient is set by defining a temperature for L0 and a temperature for L10. The corresponding lines are marked on the thermoblock. This distance between L0 and L10 is effectively used for the separation. For the evaluation of stained gels we have included a plastic film where lines L0 to L10 are indicated. The gel is placed on the film and the position of the bands can be identified in correlation to the temperature lines (see section 6.2.7).

4.2 Design of TGGE experiments

There are 3 steps in the setup of a new TGGE experiment:

1) Design of the PCR fragment

2) Identification of the correct temperature gradient

3) Parallel analysis of multiple samples

4.3 Design of DNA fragment for TGGE

The design of the DNA fragment is an important step for successful TGGE. Starting with the gene fragment of interest PCR primers should be designed with a conventional computer program. The melting behaviour of the resulting fragment should then be checked with the Poland software. It is essential that the DNA fragment shows different melting domains. If there is only one single melting domain, an artificial higher melting domain (called GC clamp) must be added during PCR.

4.3.1 Poland analysis

The melting profile of a DNA fragment can be analyzed with a computer program. The Poland software calculates the melting behaviour of a DNA fragment according to its base sequence. This software is free accessible via the internet.

(http://www.biophys.uni-duesseldorf.de/POLAND/poland.html).

How to perform a Poland analysis

- Open start page (URL see above)
- 1) enter a name for the query
- 2) copy / paste DNA sequence in the sequence window
- 3) choose the Tm plot (de-activate all other plots)
- 4) submit query
- 5) retrieve Tm plot (melting curve)

Poland service request form

The Poland server will calculate the thermal denaturation profile of double-stranded RNA, DNA or RNA/DNA-hybrids based on sequence input and parameter settings in this form.

NEW: Thermodynamic parameters set for dsDNA in 75 mM NaCl (Blake & Delcourt) added.

Calculation is based on **Poland**'s algorithm in the implementation described by **Steger**.
Graphics results are directly sent to your WWW client.

1) **Sequence title line:**

2) **Sequence:**
(plain format;
no numbers;
max. 1000 nts;
min. 5 nts)

Mismatched positions:
(comma-separated numbers)

Thermodynamic parameters:

Dissociation constant ß:

Oligonucleotide
(ß is function of seq.length)

Long double strand
(default: ß=1.0E-3/M)

Strand concentration:
(default: 1.0E-6 M)

Temperature range:

Low temperature limit:
(default: 40.0 °C)

High temperature limit: (default: 110.0 °C)

Temperature step size:
(default: 2.0 °C)

3) **Which graphics do you want:**

T_m(p=50%) plot	3d plot	Mobility plot	Melting curve	Diff. melting curve
☑	☐	☐	☐	☐

Graphics size:
(GIF format)

4) **Click here to** submit**, or**

Click here to Zurücksetzen **the form to defaults.**

The Tm plot (second order, red color) shows the melting profile of the DNA fragment according to the base sequence. The ideal fragment shows at least two distinct melting domains. Note that mutations can be detected in all but the highest melting domain. This means that in a DNA fragment with two melting domains, mutations can only be detected in the lower melting domain.

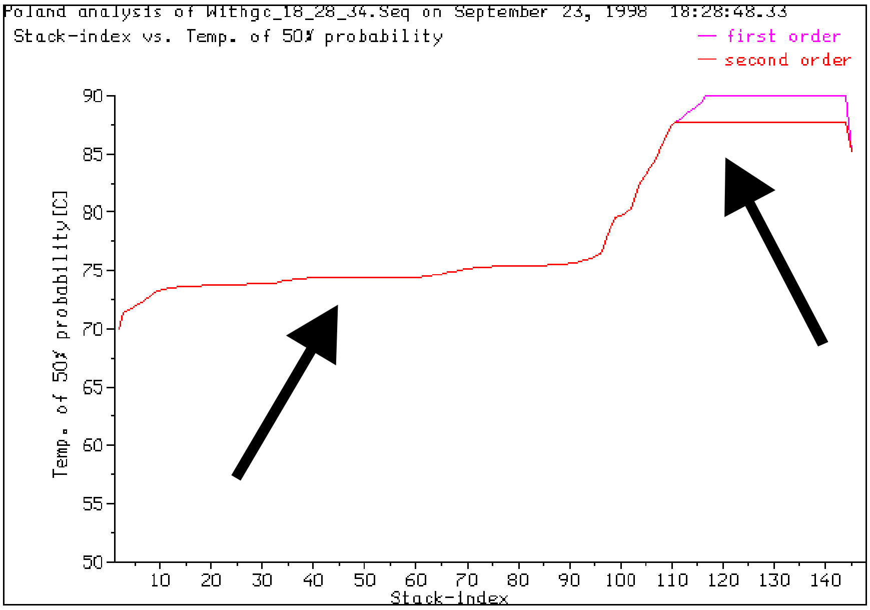


Figure 4: Tm plot of a 140bp DNA fragment resulting from Poland analysis. The second order curve (red color in the original) shows two different melting domains.

If the fragment consists of a single melting domain only, or if you want to scan the entire fragment for mutations, add a so called GC clamp to one end of the PCR fragment.

4.3.2 GC clamps

A GC clamp is an artificial, high melting domain which is attached to one end of the fragment during PCR. The name “GC clamp” implies that this short stretch will hold the DNA fragment together, preventing a dissociation into the single strands at higher temperatures. The optimum location for the GC clamp at the PCR fragment (5´ or 3´) can be easily checked with the Poland software. Copy / paste the GC sequence to either side of your sequence and repeat Poland analysis. In the following box you will find different examples for a GC clamp.

short GC-clamp (23 bp):	**cccgc cgcgc cccgc cgccc gcc**

long GC-clamp (40 bp)44:	**cgccc gccgc gcccc gcgcc cggcc cgccg ccccc gcccg**
long GC-camp (39 bp)45:	**ccccg ccccc gccgc ccccc ccgcg cccgg cgccc ccgc**

To integrate a GC clamp into a PCR fragment, one of the two primers has to be modified. The non-specific GC sequence is added to the 5´-end of the primer. Thus the GC sequence is incorporated in the fragment during PCR. Prior to order primers check the melting behaviour of the PCR fragment with GC clamp by Poland analysis (see chapter 4.3.1). Figure 5 shows a Tm plot for a PCR fragment with GC clamp either at the 5'-or 3'-end.

GC-clamp at 5´end **GC-clamp at 3´end**

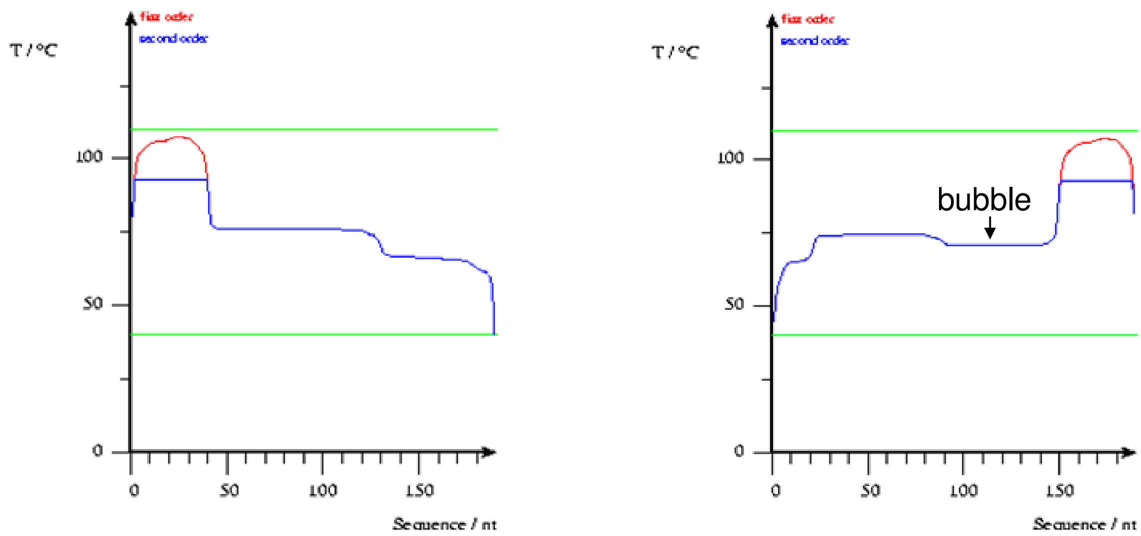


Figure 5: Tm-Plot of a PCR fragment with GC clamp at the 5'-end or at the 3'-end. Whereas the fragment with 5'-GC clamp has domains that melt consecutively and is well suited for TGGE analysis the fragment with 3'-GC clamp has a domain in the middle that melts at lower temperature compared to the neighbouring domains and is therefore leading to bubble formation. Thus the 3'-GC clamp for this fragment will not work for TGGE analysis.

4.3.3 Chemical clamp with Psoralen (Furo[3,2-g]coumarin, $C_{11}H_6O_3$)

In addition to "clamping" a fragment with an artificial high melting domain it is as well possible to covalently fix the end of a PCR fragment. To achieve this, one of the primers carries a Psoralen molecule. Psoralen is a high reactive group when exposed to UV radiation. Thus it is possible to covalently close one end of the PCR fragment. The optimal primer sequence may be 5'(Pso)pTaPpnpnp.....3', given the preference of Psoralen for binding between TpA and ApT pairs 13,46,47. Crosslinking of the PCR product is done e.g. in a flat-bottom microtiter plate using a 365 nm UV source. Working with small volumes it may be necessary to minimize evaporation by cross-linking at 4 – 10°C. The yield is not affected by temperature. The distance of the sample from the UV source affects the yield. 15 min at 0.5 cm distance of the sample from an 8 W UV lamp is sufficient.

4.3.4 Use of SSCP primers

In many cases primer from SSCP may be used for TGGE analysis. Nevertheless, we highly recommend to check SSCP fragments including primer sequences via Poland analysis first since the fragment might not be suited for TGGE analysis. For example, a mutation occurring in the high melting domain might lead to non-interpretable results. If there is only one melting domain add a GC clamp to one of the primers (see section 4.3.2)

4.4 Find correct temperature gradient

Poland analysis gives the first indication, which temperature gradient should be applied for parallel analysis of multiple samples. Under experimental conditions separation is performed in the presence of high concentrations of urea. Urea lowers the melting temperature of the DNA. This is important because gel electrophoresis at very high temperatures may lead to partial drying of the gel, resulting in a disturbed separation pattern. Therefore it is necessary to identify the optimum temperature gradient under experimental conditions.

To identify the optimum temperature gradient the DNA fragment is separated in a perpendicular TGGE. This means the temperature gradient is perpendicular to the migration of samples (see section 6.2.5). Thus the migration of a fragment can be checked simultaneously at different temperatures in a single run. If the PCR fragment has been designed properly the separation in a perpendicular temperature gradient leads to a distinct melting curve (see Figure 6)

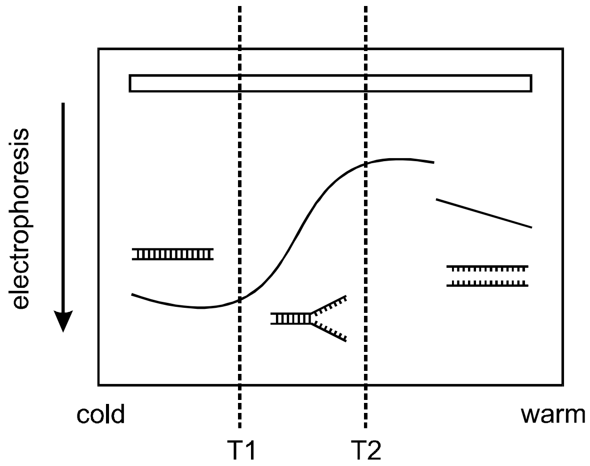


Figure 6: Identification of the optimum temperature gradient in a perpendicular TGGE. At low temperature (below T1) DNA migrates as a double strand (left side). At intermediate temperature (between T1 and T2) the DNA opens at one side, the partial double strand is increasingly slowed down. Above T2 the DNA separates into the single strands.

At T1 the double strand starts to melt and forms a branched structure. At T2 the partial double strand separates irreversibly into the single strands. Analysis of samples in parallel TGGE should be performed precisely in this temperature range between T1 and T2.

How to identify the optimum temperature range from a perpendicular gel:

Place the stained gel on the plastic film with the printed lines (l0 to L10). Identify the line where the double strand starts to melt (T1) and the line where the double strands separates into the single strands (T2). For the calculation of temperature at the corresponding lines see section 6.2.7.

4.5 Parallel analysis of multiple samples

After identification of T1 and T2 in a perpendicular TGGE this temperature gradient is spread over the whole block for parallel analysis.

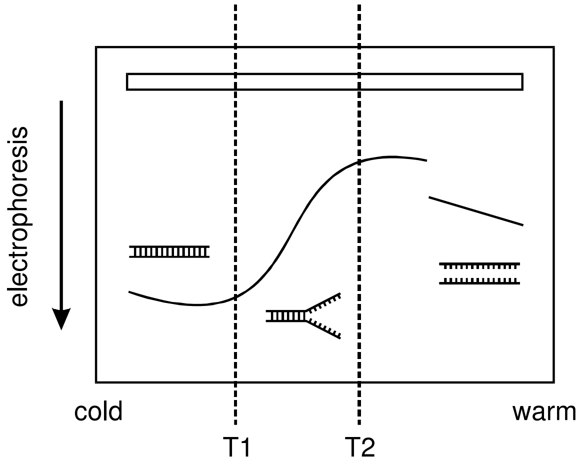


Figure 7: Application of T1 and T2 in a parallel gel.

Note: The DNA fragments are separated by their melting behavior. They can be distinguished as soon as the fragments begin to melt, i.e. they form a fork like structure (temperature higher than T1). During electrophoresis the fragments should not separate into single strands. This is an irreversible transition resulting in diffuse bands.

Note: If there are only small differences in the migration of different samples, perform a heteroduplex analysis (see chapter 9.3)

5. Sample preparation

5.1 Purity of samples

Due to the high sensitivity of the staining procedure after TGGE it is recommended to use purified DNA, RNA or protein samples. Any impurities might be misinterpreted after TGGE, thereby making the analysis of gels difficult. Nevertheless it is possible to use even crude mixtures for TGGE analysis.

PCR-amplified DNA fragments usually can be analyzed without further purification. Please note that the presence of high amounts of nonspecific, secondary PCR products may result in difficulties with interpretation of band pattern, melting profile, etc. For example, in parallel TGGE, nonspecific bands with a higher molecular weight than the specific PCR product may be misinterpreted as heteroduplices, or analogs with lower thermal stabilities. Therefore, prior to TGGE check the PCR product in a conventional agarose gel. If necessary, purify your specific PCR product, e.g., by agarose gel electrophoresis and subsequent gel extraction.

5.2 Sample preparation for direct DNA analysis

1 volume of DNA/RNA samples is mixed with 1 volume of TBE or Na-TAE loading buffer or with 0.1 volume of the total loading volume ME loading buffer (see Appendix). The resulting mixture is loaded directly on to the polyacrylamid gels. Be sure that the slots are filled up to maximum (if necessary, add 1x loading buffer to fill up the slots to maximum).

In case of low-concentration samples we recommend to prepare 5x conc. loading buffer. 0.2 volume of this concentrated loading buffer is mixed with 0.8 volumes of the sample and loaded onto the gel.

5.3 Denaturation / Renaturation for heteroduplex analysis of DNA

Mix sample with equal amount of standard DNA and heat to **95°C for 5 minutes (denaturation)**. Then let slowly cool down to **50°C (renaturation)**. This can be done by programming a thermocycler to 94°C for 5 minutes and then 50 °C for 15 minutes with a ramping rate of –0.1°C/second. The sample is then loaded directly to the gel. In order to achieve the recommended loading volumes for diagonal or perpendicular TGGE , the sample volume should be adjusted with running buffer.

Important: Do not denature/renature DNA for community profiling (see chapter 6.1.3)!

6. Operating

6.1 Casting of gels

6.1.1 Assembly of the gel cassette

There are two different types of gels for TGGE. For perpendicular gels one long slot is used to separate one sample over a broad temperature range (use glass plate with slot former, 024-228). For parallel gels samples are applied with an applicator strip. For use with this strip a gel without slots is casted (use glass plate without slot formers, 024-227), then the applicator strip is applied onto the gel just prior to run. This silicone applicator strip contains 32 holes and is simply placed on top of the gel. Samples are loaded in the holes. After turning on voltage samples diffuse into the gel and migrate along the gel matrix.

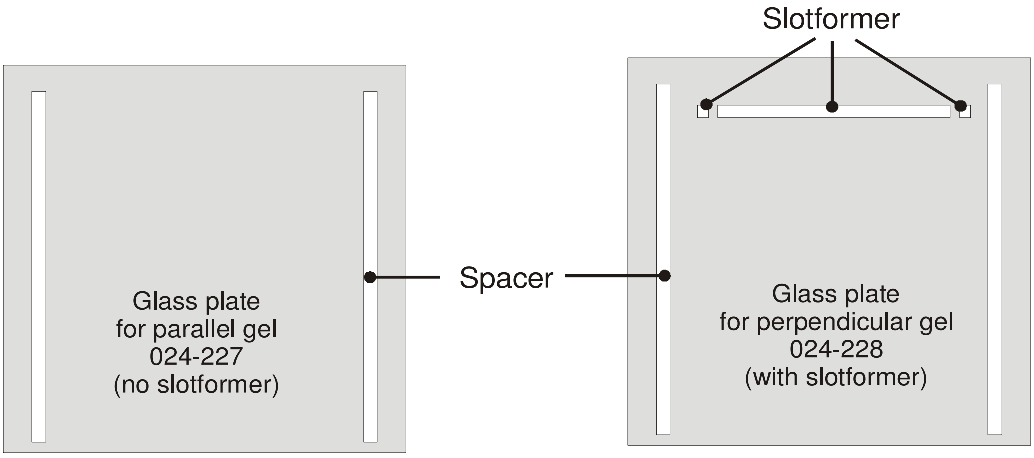


Figure 8: Glass plates for casting parallel (left) or perpendicular gels (right).

The gel cassette consists of one glass plate with spacers (024-227 or 024-228), glass plate without spacers (024-221) and a silicone sealing (024-230). The sandwich is fixed with 9 metal clamps.

Alternatively the glass plate with spacers and slots (024-229, not supplied) can be used to assembly the gel cuvette.

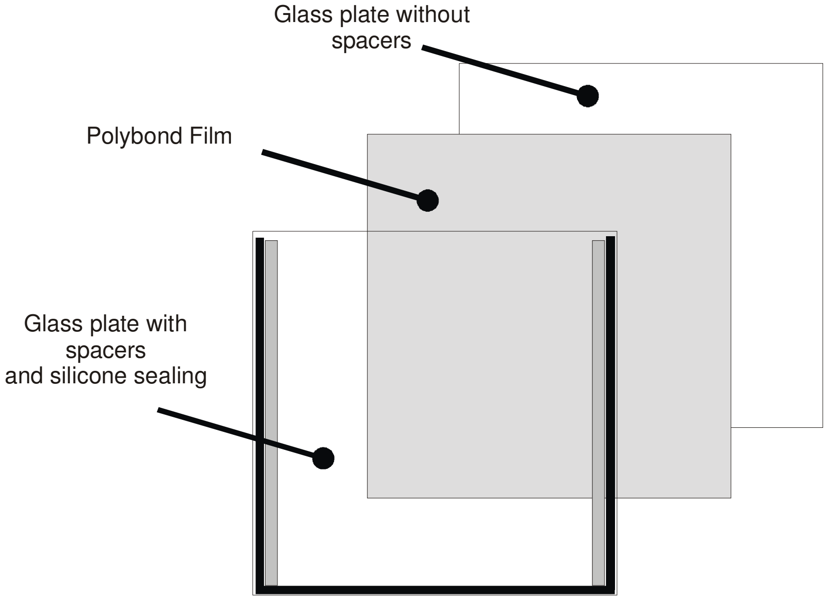


Figure 9: Setup of glass plate sandwich

Note: The gel is poured on Polybond film to optimize temperature transition between block and gel. The gel is bound covalently to this hydrophilic Polybond film (024-234).

Note: The gel sticks to the Polybond film throughout the whole procedure, including staining.

1) Clean both glass plates with 70% ethanol and a soft tissue.

2) Apply approx. 2 ml of gel repellent solution (Acryl Glide, Amresco or Repel silane, Sigma) on the glass plate **WITH SPACERS** and spread it with a soft tissue

3) Wait for 2 minutes
Polish glass plate with a soft tissue

Note: Do not apply Acryl-Glide onto the spacers, because this can lead to leakage of the gel cuvette.

4) Put polybond film (024-234) on the glass plate **WITHOUT SPACERS.**

5) Attach polybond film by carefully wiping it with a soft tissue. The film should attach uniformly to the glass plate. To improve contact between glass plate and film a drop of water may be applied behind the Polybond film.

Note: The support film may be fixed along the upper side of the cover glass plate with ordinary adhesive tape. This way no gel solution can accidentally get behind the support film.

6) Place silicone sealing around spacers.

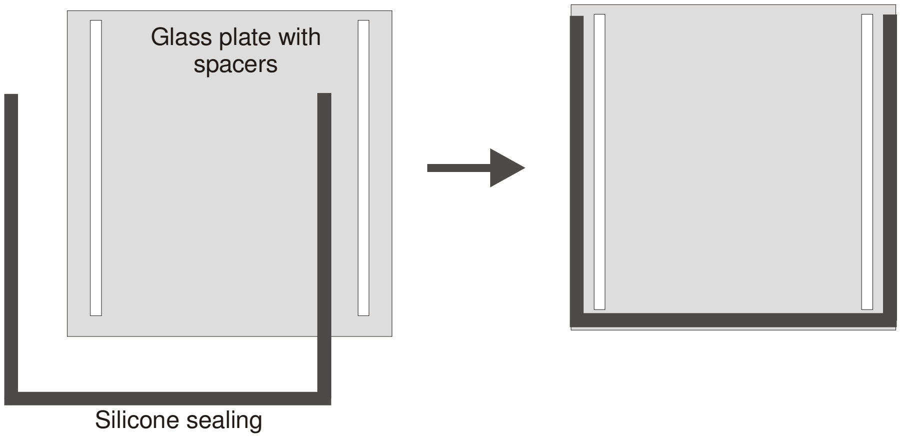


Figure 10: Assembly of glass plate and sealing

7) Assemble gel sandwich and fix it with 3 metal clamps on each side.

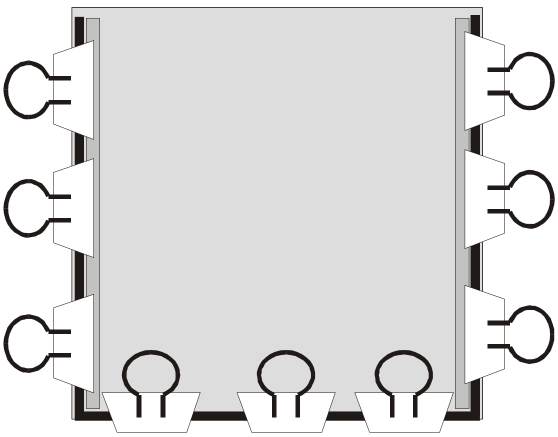


Figure 11: Final setup of the gel cuvette.

Note: the clamps should be placed directly on the spacers.

8) Set gel sandwich upright on the 3 bottom clamps (see Figure 11)

6.1.2 Preparing gel solution for mutation analysis

The choice of the buffer system has a strong impact on TGGE analysis. Concentration of salt and denaturing agents (urea or formamide) strongly affects the melting temperature of DNA and proteins. In general, urea is used for the separation of nucleic acids in a concentration between 7 and 8M. Urea reduces the melting temperature and thus enables a separation at lower temperatures (which is favourable, because at higher temperatures the gel tends to dry out). To further reduce the melting temperature (deionized) formamide may be used in concentrations of up to 20%. The most popular buffer systems for TGGE are TBE, TAE and MOPS (see also chapter 17.2). In the following paragraph a standard protocol for the TAE buffer system is listed. Please note, that the buffer system should be adapted to each special kind of application. In chapter 6.1.3 the gel composition for the separation of fragments from mixed populations is given.

For one TGGE MAXI gel prepare 50 ml gel solution

TAE gel composition final concentrations	stock solution	for 50 ml	for 100 ml
Acrylamide [8%]	40 % (37,5 :1)	10 ml	20ml
Urea [8M]	solid	24 g	48 g
TAE [1x]	10 x	5 ml	10 ml
Glycerol [2%]	40%	2,5 ml	5 ml
Adjust with aqua bidest		to 50 ml	to 100ml

- Stir solution at 50℃ until urea is completely dissolved.
- Carefully degas gel solution
- let cool down to room temperature and start polymerization with

APS	10 %	80 µl	160 µl
TEMED	100%	45 µl	90 µl

- Load gel solution in a syringe and attach a 0.4µm or 0.25µm sterile filter
- Pour gel through sterile filter into the glass sandwich.

Protocols for mutation analysis are given in chapter 7.3.

6.1.3 Gel preparation for analysis of mixed bacterial populations (genetic fingerprint)

For genetic fingerprinting primers against 16SrRNA genes are used. Depending on the complexity of the sample and the primer design quite a high number of PCR fragments can arise from one sample. In this regard there are two major differences to mutation analysis: 1) the number of fragments per lane is much higher 2) the amount of DNA per band is lower. To separate all the many fragments a rather flat gradient is used which has to be optimized very carefully. For detecting low amounts of DNA a clear staining background is essential. The silver staining protocol (chapter 8.1.) has been optimized for gels with high content of urea and formamide and thus achieves high sensitivity at low background.

For microbial diversity analysis we recommend the following conditions. However, these parameters can only be the starting point for individual optimization.

TAE gel composition final concentrations	stock solution	for 50 ml	for 100 ml
Acrylamide [6%]	40 % (37,5 :1)	7,5 ml	15 ml
Urea [8M]	solid	24 g	48 g
TAE [1x]	10 x	5 ml	10 ml
Glycerol [2%]	40%	2,5 ml	5 ml
Deionized formamide [100%]	20%	10 ml	20 ml
Adjust with aqua bidest		to 50 ml	to 100ml

- Stir solution at 50℃ until urea is completely dissolved.

- Carefully degas gel solution
- let cool down to room temperature and start polymerization with

APS	10 %	80 µl	160 µl
TEMED	100%	45 µl	90 µl

- Load gel solution in a syringe and attach a 0.4µm or 0.25µm sterile filter
- Pour gel through sterile filter into the glass sandwich.

Protocols for mutation analysis are given in chapter 7.4.

Important: Do not denature/renature the DNA fragments prior to electrophoresis!

Tip: Formamide can be a "tricky" component since it doesn't have the highest stability in general. You might even leave formamide in the experiments - a test with and without formamide will show whether the fragments also become separated without this component at the same resolution. The preparation 20 ml aliquots that are stored at -20 °C is recommendable.

6.1.4 Pouring gels

1) Pour gel solution slowly into the sandwich. Avoid bubbles!
2) Let polymerize for approx. 3 h at room temperature

Note: Polymerized gels may be stored for 3 days or even longer at **room temperature**. Remove clamps and wrap gel sandwich including glass plates in wet paper towels. Store the sandwich in a tight plastic bag.

Note: Prepare electrophoresis unit prior to disassembling the gel cassette. The gel should not be exposed to the air for extended periods since this may lead to drying of the gel.

3) After polymerization remove clamps. Remove the glass plate without spacers by sliding the glass plate away from the rest of the sandwich (if you have fixed the support film with adhesive tape, remove or cut tape first). The gel must stick on the support film.
4) Remove gel together with support film carefully from the glass plate with spacers. Be careful not to damage the slots.

6.2 Electrophoresis

6.2.1 Electrophoresis conditions

The electrophoresis unit of the TGGE System has been designed to accommodate TGGE and all related applications like CTGE, TTGE and SSCP without cumbersome changes. The buffer tanks can be positioned in two orientations, allowing a temperature gradient parallel or perpendicular to the electrophoresis direction (see section 6.2.5 and 6.2.6).

Electrophoresis conditions in general depend on

- the kind of sample, e.g. protein, nucleic acid, fragment size
- the kind of application, e.g. parallel or perpendicular TGGE
- the sample preparation, e.g. high salt or low salt preparation,

- the buffer system.

Any recommendations should be regarded as guidelines to start with. Further improvement of the analysis should be done by adjusting the run conditions to individual needs.

Important: The controller is designed to control voltage rather than amperage (set [mA] and [Vh] to maximum values).

m can be set up in the controller

6.2.2 Setup electrophoresis unit

The Biometra TGGE system is a horizontal electrophoresis system. The buffer bridges to the gel are established by layering one side of each buffer wick (024-215) on the gel and submerging the other in the buffer inside the tank. To protect the gel from drying, it is covered with a gel cover film (024-232). The complete setup, consisting of gel with cover film and buffer wicks, is covered with the gel cover plate. The gel cover plate has two sealings and fits tightly onto the thermoblock. It holds the buffer wicks in place and helps to build a humidity chamber around the gel. This is important to prevent evaporation during the run.

⚠	**Important:** Never run a gel without gel cover plate. (This could lead to massive condensation under the safety lid. Danger of electric shock.)

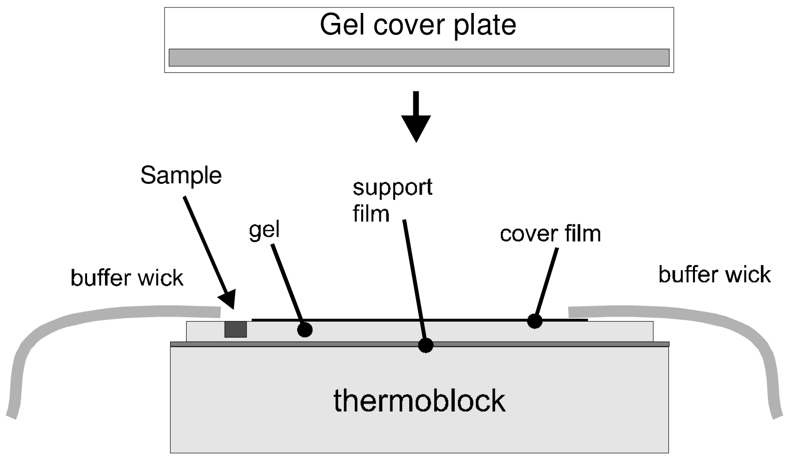


Figure 12: Set up of the gel for electrophoresis

6.2.3 Prior to assembly of the electrophoresis unit

Note: Be sure to have everything on hand, to avoid extended handling times. Don't let the disassembled gel dry, during setup of the electrophoresis system.

Prepare:

➲ parallel or perpendicular gel (see section 4.2)

➲ samples in loading buffer (for sample preparation see chapter 5)

- ➲ 1.000 ml running buffer (for recipes see chapter 17.2)
- ➲ 2 re-usable buffer wicks (024-216)
- ➲ 1 cover film (024-232)
- ➲ thermal coupling solution (0.1% Triton X-100 or 0.1% Tween 20, degas carefully)
- ➲ gel cover plate

6.2.4 Gel setup for electrophoresis

1) Adjust electrophoresis chamber with the 4 levelling feet.

2) Fill 500 ml **running buffer** in each buffer tank (check orientation of the buffer tanks: parallel or perpendicular TGGE! see section 6.2.5 and 6.2.6).

Note: Wipe off any spilled buffer from the electrophoresis unit. Never run device if buffer has been spilled.

3) Soak 2 **buffer wicks** with running buffer

4) Disassemble **gel sandwich**. Carefully clean backside of the gel support film with a soft tissue.

5) Apply not more than 1.5 ml of **thermal coupling solution** (0.1% Triton X-100 or 0.1% Tween 20) on the thermoblock

Note: The volume of coupling solution should be as small as possible. Excess coupling solution leads to an irreproducible temperature distribution under the gel. The result is a wavelike migration front and poor separation of fragments.

6) Place the **gel on the thermoblock**. The thermal coupling solution should spread over the whole block. Avoid formation of bubbles. Wipe off any residual coupling solution along the edges of the gel support film.

Note: The thermal coupling solution is essential for efficient heat transfer from block to gel. If bubbles are entrapped under the gel support film, remove support film with gel from the block and place it back again on the block.

7) Apply applicator strip for sample loading

8) Cover the gel just beneath applicator strip with a cover film.

9) Attach pre-soaked **buffer wicks** on top and bottom of the gel.

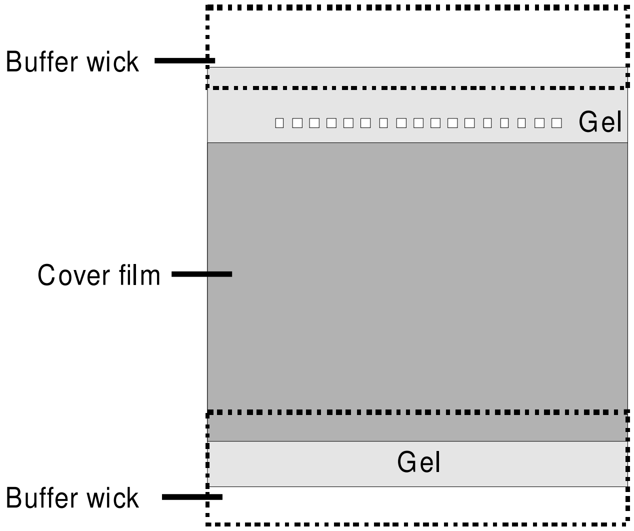


Figure 13: Setup of gel, cover film and buffer wicks.

10) Load **samples** (approx. 5µl each for parallel gel with 32 slots, approx. 200µl for perpendicular gel with)

Note: Be careful not to touch the samples with the buffer wick! Otherwise the samples will diffuse into the wick.

11) Attach **gel cover plate** (the cover plate should have contact to the wicks, but must not squeeze gel or wicks).

12) Close **safety lid** and start **run**.

Note: For parallel TGGE let temperature gradient equilibrate for approx. 10 minutes, then start main run. This step may be omitted for a perpendicular gel.

6.2.5 Perpendicular TGGE

In perpendicular TGGE one sample is separated over a broad temperature range. This application is mainly used to check the melting behaviour of a sample (see section 4.4). For casting of the gel use a glass plate with one large slot former (024-228). The temperature gradient must be orientated perpendicular to the migration of the sample. The buffer tanks must be positioned as described in Figure 14.

The migration of DNA / RNA molecules is indicated by the arrow on the safety lid of the electrophoresis unit.

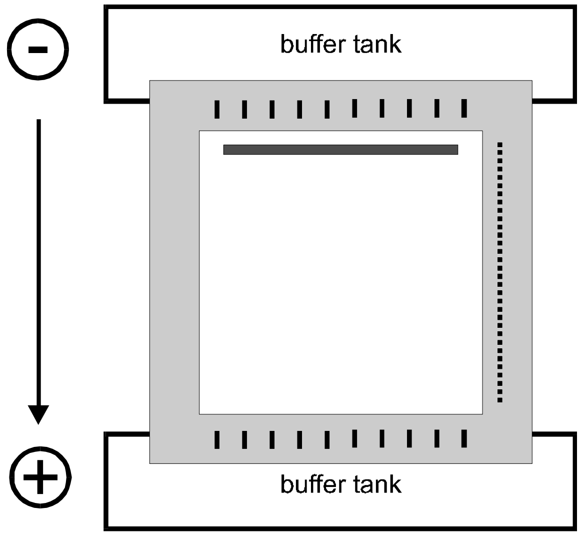


Figure 14: Positioning of buffer tanks for perpendicular TGGE. Be sure that the direction of electrophoresis is perpendicular to the temperature gradient. The temperature gradient is indicated by the lines on the edge of the block.

6.2.6 Parallel TGGE

In parallel TGGE multiple samples are separated along the temperature gradient. For gel casting gel use glass plate with spacers (024-227). The buffer tanks for parallel TGGE must be positioned as depicted in Figure 15.

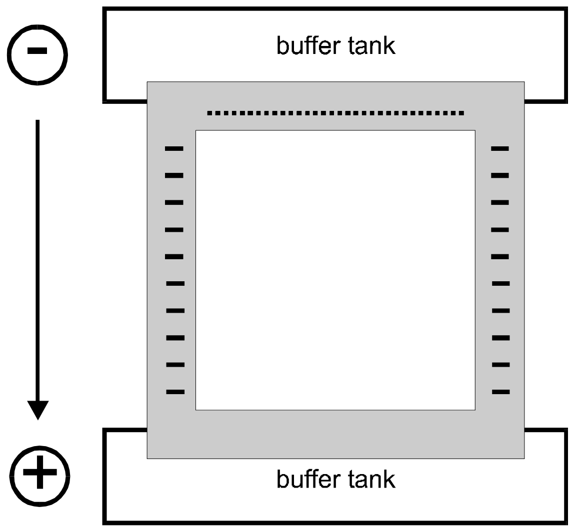


Figure 15: Positioning of buffer tanks for parallel TGGE. Be sure that the direction of electrophoresis is parallel to the temperature gradient. The slots of the gel should be at the same side as the markings on the block.

Note: For a parallel TGGE a 10 minutes equilibration of the temperature gradient may be included after the pre-run.

6.2.7 How to identify the optimum temperature range from a perpendicular gel

The theoretical background for the separation of DNA fragments in a perpendicular gel is described in section 4.4.

Place the stained perpendicular gel on the plastic film with the printed lines (L0 to L10). Identify the line where the double strand starts to melt (T1) and the line where the double strands separates into the single strands (T2).

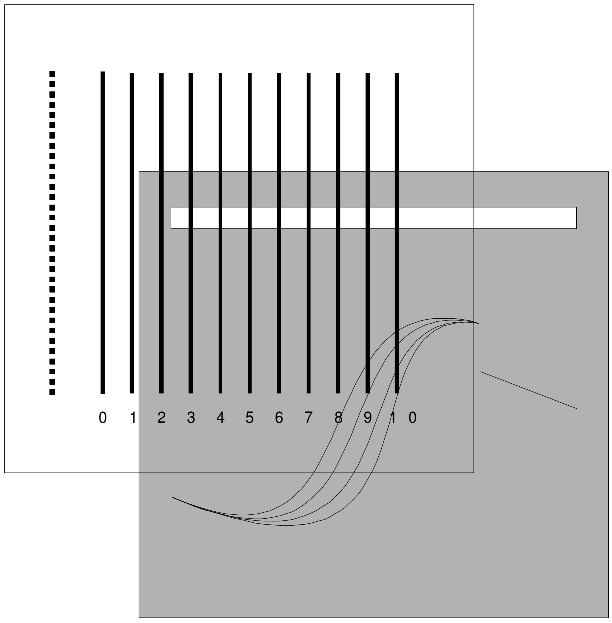


Figure 16: The calculation of the corresponding temperatures is simple, since there is a linear temperature gradient between L0 and L10 (i.e. the temperature increment from one line to next line is always the same).

Calculation: Divide range of gradient by ten, this is the temperature increment from one line to the adjacent lane.

Example: calculation of temperature at line 6 (L6) in a temperature gradient from 40℃ (L0) to 60℃ (L10)

- **subtract** temperature at L0 from temperature L10 (range of gradient: 60-40℃ = 20℃)
- **divide** temperature by 10 (increment from line to lane: 20℃/10 = 2℃)
- **multiply** increment by 6 (6 increments from L0 to L6: 12℃)
- **add** this value to the temperature at L0 (40℃ + 12℃)

result: temperature at L6 is 52℃

7. Programming the TGGE controller

7.1 General overview

All parameters of the run are controlled by the TGGE system controller. This includes electrophoretic parameters (voltage, amperage, time) as well as control of the temperature gradient.

Note: Each program can consist of several steps. Thus you can program pre-run, equilibration and main run in the same program (see also section).

7.1.1 Create / edit program

Main screen

L0: 20.0℃   L10: 20.0℃
maxi block off

A? BElpho Cprograms D+

Press C [programs] to enter the programming mode

program no:

Alist Bdel Cquit Denter

7.1.2 Select program

Enter a program directly by number or press A [list] to view a list of the existing programs.

0 test 1 maxi

1 parallel mini
2 empty
A↑ B↓ C quit D enter

Scroll through the list with A↑ B↓ and accept highlighted program with D [enter].

Note: Each program depends on the type of TGGE system (mini or maxi). The type (mini or maxi) for which the program was written, is displayed behind the program name. Programs can only be written /edited in the respective mode. Programs that are created in the maxi mode are automatically saved as maxi programs and can only be run with the maxi system (and vice versa).

To set type of system (mini or maxi) see section 7.2.4.

7.1.3 Name program

Each program is specified by name and a program number. To facilitate retrieval of a program, you can enter a name for each program existing of letters, numbers and symbols.

name:>   <

ABCDEFGHIJKLMNOPQRST
UVWXYZ –()αδ/,〈〉&+.%
A➔ BABC C quit D enter

Press B [ABC] to enter the mode for the selection of letters.

name:>   <

ABCDEFGHIJKLMNOPQRST
UVWXYZ –()αδ/,〈〉&+.%
A🡐 B➔ C quit D enter

Move to the desired letter with A [🡐] and B [➔]. Accept highlighted letter with D [enter].

name:>test   <

ABCDEFGHIJKLMNOPQRST
UVWXYZ –()αδ/,〈〉&+.%
A🡐 B➔ C quit D enter

If the program name is complete, confirm name with D [enter]. In the following screen you can set the temperatures for the gradient block.

7.1.4 Enter temperatures for the gradient block

1:L0:   L10:

A?   B   C quit D ➔

Enter temperature for L0 and accept with D [enter].

1:L0:30.0   L10:

A? Bdelete Cquit Denter

Enter temperature for L10 and accept with D [enter].

1:L0: 30.0   L10: 70.0

ok?
A   B no   C quit D yes

Confirm settings with D [yes]. If settings are not correct press B [no] and repeat entry of temperature settings.

After you have confirmed the temperature settings, the following screen is displayed. Here you can enter all parameters for electrophoresis.

7.1.5 Enter electrophoresis parameters

1:L0: 30.0   L10: 70.0
  time:
El: 0V 500mA 50W
A?   B V*h   C quit D ➔

Enter time for electrophoresis and accept with D [enter].

Note: There is a convention on how time settings are entered in all BIOMETRA instruments:

hours ● minutes ● seconds

If you enter a number without "dot" this value will be interpreted as seconds ("300" => 5 minutes). To program minutes enter a "●" after the number of minutes. To enter hours enter ● ● after the number. You can also enter any combination of hours, minutes and seconds. Example: for 1 hour, 30 minutes, 20 seconds enter 1● 30 ● 20.

The time values will be displayed in the following format: 00 m 00s

Accept time setting with [D enter]

1:L0: 30.0   L10: 70.0
  time: 10m 0s
El: 0V 500mA 50W
A? Bdelete C quit D enter

Enter Voltage and accept with D [enter]

1:L0: 30.0   L10: 70.0
  time: 10m 0s
El: 300V 500mA 50W
A? Bdelete C quit D enter

Note: The values for amperage [mA] and wattage [W] are set to maximum level as default. If you enter lower values, these parameters may become limiting during electrophoresis.

Accept default settings for voltage and wattage with D [enter] or enter different values.

Note: We recommend to control electrophoresis by constant voltage rather than by constant amperage (set [mA] to maximum value, respectively accept default value).

Note: Each program can consist of several steps. Thus it is possible to program complex protocols including a pre-run, a pause for handling of the gel and the main run..

In the following screen you can program a second step for your protocol.

2:L0:   L10:

A? B C quit D ➔

If you do not want to program another step, accept program with C [quit].

The program name, number of steps and the total run time is displayed.

program no. 8
pgm end: 1 step(s)
run time: 0h10m 0s
A? B C quit D ➔

7.1.6 Start electrophoresis

Main screen:

L0: 20.0℃ L10: 20.0℃
maxi block off

A? BElpho Cprograms D+

To start a program press B [Elpho]

start program:

Alist Bdel Cquit Denter

Enter program number or choose a program from the list with A [list].

start program: 8

Alist Bdel Cquit Denter

Confirm program number with D [enter].

The program starts and parameters of gradient block and electrophoresis are displayed. During temperature equilibration of the gradient block the elapsed time is displayed.

Note: Electrophoresis starts as soon as the set temperature in the block is achieved.

L0: 30.0℃ L10: 70.0℃
hold: 1 2m12s 11.4Vh
El: 300V 8mA 20.3W
A? BElpho Cprograms D+

7.1.7 Stop/pause electrophoresis

L0: 30.0℃ L10: 70.0℃
hold: 1 2m12s 11.4Vh
El: 300V 8mA 20.3W
A? BElpho Cprograms D+

To stop/pause the active program press B [Elpho]

program 8 test
pause ?
stop ?
A? Bpause Cquit Dstop

Press B [pause] to pause program

Press D [stop] to stop program

Press C [quit] to return to the active program.

7.1.8 View temperatures of the gradient

L0: 30.0℃ L10: 70.0℃
hold: 1 2m12s 11.4Vh
El: 300V 8mA 20.3W
A? BElpho Cprograms D+

To display the temperatures in the block during a run press A [?]:

L0 : 30.1
L1 : 34.2

L2 : 38.3
A↑ B↓ C quit D enter

You can scroll through the different lines with A↑ B↓.

L6 : 54.8
L7 : 58.9
L8 : 62.9
A↑ B↓ C quit D enter

Note: There is a difference is the number of lines depending on the type of TGGE system (mini or maxi) that is installed. In the maxi mode 10 different temperatures are displayed (L0 to L10) in the mini mode 5 different temperatures are displayed (L1 to L6).

7.2 Special functions

Main screen

L0: 20.0℃ L10: 20.0℃
maxi block off

A? BElpho Cprograms D+

Press D [+] to enter the menu for special functions

1 print programs
2 signal

3 language
A↑ B↓ C quit D enter

Scroll through the list with A↑ B↓.

7.2.1 Print programs

Connect controller to a dot matrix printer. Select option 1 in the above menu and confirm with D [enter].

7.2.2 Select / de-select signal

Select option 2 in the special functions. Press A [on] to activate the signal, press B [off] to inactivate signal.

7.2.3 Select language

Select option 3 in the special function screen. Choose between German and English

7.2.4 Set block type

Select option 6 in the special function screen. Choose between mini and maxi system.

Note: The selection of the TGGE system type (mini or maxi) is saved together with each individual program. Programs that have been written in the MAXI mode can only be edited and run in the maxi mode. Programs that have been written in the mini mode can only be edited and run in the mini mode.

7.3 Electrophoresis protocols for mutation analysis

7.3.1 Electrophoresis protocol for perpendicular TGGE*

Step 1:

L0: 20.0 L10: 20.0 time: 10m 0s El: 300V 500mA 50W A? B V*h C quit D →	Pre-run

Step 2:

L0: 30.0 L10: 70.0 time: 10m 0s El: 0V 500mA 50W A? B V*h C quit D →	Equlibration phase of the gradient

Step 3:

L0: 30.0   L10: 70.0   Main run
time: 3h 30m 0s
El: 300V 500mA 50W
A? B V*h C quit D ➔

* all information provided has to be considered as starting point. Further adaptations might be necessary

7.3.2 Electrophoresis protocol for parallel TGGE*

Step 1:

L0: 20.0   L10: 20.0   Pre-run
time: 10m 0s
El: 300V 500mA 50W
A? B V*h C quit D ➔

Step 2:

L0: XX.X   L10: YY.Y   Equilibration phase of the gradient§
time: 10m 0s   XX.X = start of DNA melting* minus 5 °C§§
El: 0V 500mA 50W   YY.Y = end of DNA melting* plus 5 °C§§
A? B V*h C quit D ➔   §determined in the perpendicular TGGE experiment
§§safety margin (might not be required)

Step 3:

L0: XX.X   L10: YY.Y   Main run
time: 4h 0m 0s
El: 300V 500mA 50W
A? B V*h C quit D ➔

* all information provided has to be considered as starting point. Further adaptations might be necessary

7.4 Electrophoresis protocols for mixed population analysis

7.4.1 Electrophoresis protocol for perpendicular TGGE*

Step 1:

L0: 20.0   L10: 20.0   Pre-run
time: 10m 0s
El: 300V 500mA 50W
A? B V*h C quit D ➔

Step 2:

L0: 30.0 L10: 70.0 time: 10m 0s El: 0V 500mA 50W A? B V*h C quit D ➔	Equlibration phase of the gradient

Step 3:

L0: 30.0 L10: 70.0 time: 16h 0m 0s El: 130V 500mA 50W A? B V*h C quit D ➔	Main run

* all information provided has to be considered as starting point. Further adaptations might be necessary

7.4.2 Electrophoresis protocol for parallel TGGE*

Step 1:

L0: 20.0 L10: 20.0 time: 10m 0s El: 300V 500mA 50W A? B V*h C quit D ➔	Pre-run

Step 2:

L0: XX.X L10: YY.Y time: 10m 0s El: 0V 500mA 50W A? B V*h C quit D ➔	Equilibration phase of the gradient§ XX.X = start of DNA melting* minus 5 °C§§ YY.Y = end of DNA melting* plus 5 °C§§ §determined in the perpendicular TGGE experiment §§safety margin (might not be required)

Step 3:

L0: XX.X L10: YY.Y
time: 16h 0m 0s
El: 130V 500mA 50W
A? B V*h C quit D ➔

* all information provided has to be considered as starting point. Further adaptations might be necessary

8. Staining of gels

8.1 Silver staining

Aside from autoradiography silver staining is the most sensitive method for detecting small amounts of DNA, RNA or proteins in polyacrylamide gels. Other staining protocols may be used, but generally exhibit less sensitivity. This must be considered in relation to the amount of DNA loaded on the gel.

All incubation steps are done in small plastic containers which are agitated on a rocking platform.

Wear non-powdered protective gloves during all steps of the silver staining protocol to avoid staining artifacts due to the high sensitivity of the staining protocol.

The quality of chemicals is essential in silver staining. Prepare solutions fresh, use only chemicals of high quality (p.a.) and fresh double distilled water.

Important: Remove the protective plastic sheets from the gel.

- Carefully remove any residual thermal coupling solution from the back of the gel (gel support film) prior to staining.
- Put the polyacrylamid gel with the gel side upwards into the staining tray. Avoid air bubbles during all staining steps.
- It's recommended to prepare at least 400 ml solution for each incubation step.
- Prepare stopping solution prior to developing.

8.1.1 Silver staining protocol

Step	Time	Solutions
Fixation	30 min	300-400 ml 10% glacial acid, 30% EtOH
Sensitization	2 x 30 min	300-400ml 30% EtOH
Washing	Rins gel 30 seconds under running water, then wash 5 x 10 min	 Fresh aqua dest
Silver Binding	30 min	400ml 0,1 % $AgNO_3$, prepare freshly add 400µl Formaldehyde (37%) prior to use
Washing	Rins 30 seconds then wash 1 min Rins again 30 seconds	Fresh aqua dest.

Developing	Until bands become visible, can take several minutes, don´t let gel unattended!	Solution 1: dissolve 2g Sodium thiosulfate ($Na_2S_2O_3$) in 10ml bidest, Solution 2: dissolve 10g Sodium Carbonate (Na_2CO_3) in 400ml bidest Add 400µl solution 1 to solution 2 Add 400µl Formaldehyde (37%)
Stopping	30 min	Dissolve 5,84g EDTA and 8g Glycine in 400ml bidest
Storage	Up to several days	10% Glycerol

8.2 Ethidium bromide-staining

Incubate the gel in staining solution (0.5 µg/ml ethidium bromide in 1 X TBE) for 30 - 45 min. Analyze under UV radiation (27).

8.3 Autoradiography

TGGE gels can also be directly exposed to x-ray films if radiolabeled samples are analyzed.

Direct exposure:

Incubate the TGGE gel for 15 min. in Fixation solution (see 8.1 Silver staining). Optional: Silver stain the gel.

Remove residual buffer from the gel. Expose to an x-ray film at room temperature.

Exposure of dried TGGE gels:

Incubate the TGGE gel for 15 min. in Fixation solution (see 8.1 Silver staining). Optional: Silver stain the gel.

Incubate the gel in 2-5% glycerol for 10 minutes to prevent the gel from cracking. Incubate an appropriate sheet of cellophane (no Saran wrap!!!!!) in 2 - 5% glycerol. Layer the cellophane on the gel. Air dry the sandwich at room temperature for one day or use a gel dryer at 50°C for at least 3h. Exposure to an X-ray film.

8.4 Elution of DNA from the TGGE gels

DNA fragments which have been separated on TGGE, for example, different alleles of one gene, can be eluted from silver-stained TGGE gel and re-amplified by PCR.

Using a Pasteur pipette, puncture the gel and extract a µl piece containing the particular DNA duplex. Incubate in 20 µl TE buffer overnight. Use a 1 µl aliquot for re-amplification.

9. Trouble shooting guide

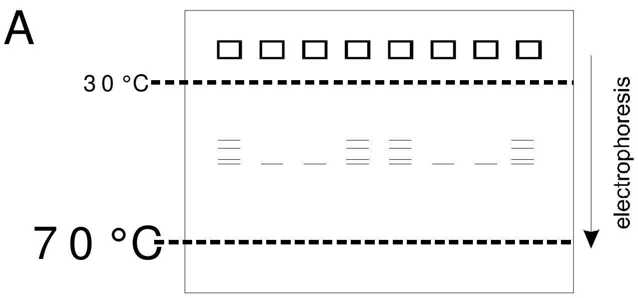
The following trouble-shooting guide may be helpful in solving any problem that you may encounter. If you need further assistance, please do not hesitate to contact your local Biometra distributor or Biometra.

Problem	Cause	Solution
Electrophoresis		
Leakage of gel cuvette	Inaccurate positioning of sealing Dust on spacers Acryl glide on spacers	Check positioning of silicone sealing, clean Spacer, do not apply Acryl Glide onto the spacers
Slotformer fall off	After heavy use this may happen from time to time	Self adhesive slot forming units (024-221) are included in the TGGE maxi system
Acrylamide solution gets behind the support film during pouring the gel	Support film is not properly attached to the glass plate	Fix polybond film with adhesive tape along the upper edge of the glass plate
Teflon film peels away from the thermoblock	Block has been cleaned with strong detergents or aggressive chemicals	Contact Biometra
No current	Amperage and Wattage have been set to “0”	Set Amperage and Wattage to maximum values (Electrophoresis should be controlled by Voltage)
Current oscillates	Coating of the thermoblock is damaged, the safety shutoff is activated	Contact Biometra
Gel interpretation		
Wavelike migration front	Temperature inhomogeneity under the gel due to excess thermal coupling solution	Use as little as possible thermal coupling solution (not more than 2ml)
No sigmoid melting curve (perpendicular TGGE)	Fragment melts completely	Perform Poland analysis Optimize primer design
No separation of hetero duplex samples (parallel TGGE)	Inappropriate fragment Wrong temperature gradient Acrylamide of poor quality	Perform Poland analysis Perform perpendicular TGGE Use only high quality chemicals (p.a.)

Irreproducible gels	Erratic temperature distribution over and under the gel	Use only minimum volume of thermal coupling solution under the gel Do not overlay gel with buffer
Silver staining		
Bad silver stain	Chemicals of poor quality Stale water Too much silver nitrate	Use only high quality chemicals Use only freshly prepared aqua bidest Refer to the staining protocol
Strong background	Insufficient washing after incubation in staining solution	Extend wash step, change water frequently
Weak staining of bands	Excessive washing after binding of staining solution	Reduce wash step after staining

9.1 Optimization of parallel TGGE

To improve separation in parallel TGGE the gradient should start directly at the temperature where the fragments start to melt (see perpendicular gel) and should be rather flat. This means there should be only a moderate temperature increase over the whole gel. Different fragments in one sample separate as soon as the first fragment starts to melt. At a certain (higher) temperature the next fragment starts to melt. In a moderate gradient, the temperature increase per centimeter is smaller than in a steeper gradient. This means, the distance between two temperatures (i.e. locations in the gel) is bigger than in a steeper temperature gradient. This results in a wider separation of fragments that melt at different temperatures (see Figure 17).



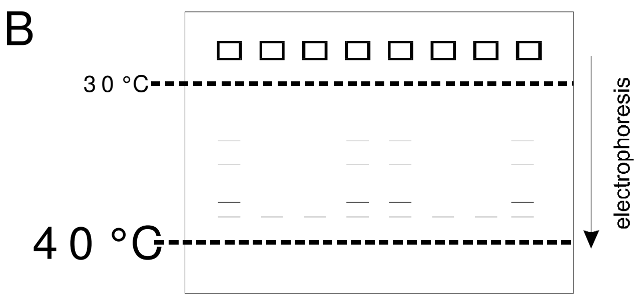


Figure 17: Parallel TGGE using a steep (A) or a flat (B) temperature gradient. With a smaller temperature gradient (30 to 40 °C, B) the separation of samples is much wider. (Note: the temperatures in this figure are only for demonstration.)

9.2 Optimization of TGGE for genetic fingerprinting of microorganisms (mixed population analysis)

Primers should be designed thoroughly to avoid unspecific PCR products. Ideally sequences of the species considered must be compared for identifying conserved regions 48. Further, the PCR cycles should be reduced to a minimum 49 while extension times should be enlarged 50 to avoid the formation of heteroduplex artefacts 52. In cases of mixed-template PCR products a low cycler number reamplification may increase specificity 52. In general, PCR products should be purified prior to TGGE analysis 51. Sometimes PCR artefacts are co-amplified which can result in smears on TGGE gels. PCR products can be purified by PCR purification kits or ideally each PCR-product is subjected to conventional agarose electrophoresis. After electrophoretic separation, for each sample the desired band can be cut from the gel and purified.

9.3 Optimisation of Heteroduplex analysis

9.3.1 Principle of heteroduplex analysis

If the difference in melting temperature between wildtype and mutant is very small, heteroduplex analysis is a rewarding approach. Heteroduplex analysis makes it very easy to distinguish between the wildtype and mutant form of a DNA fragment. The basic principle is to mix each sample with an external standard. In most cases this standard is a PCR fragment without mutations, for example amplified from the wild type. After mixing the standard DNA fragment with the PCR fragment from the sample the mixture is heated and subsequently slowly cooled down (for protocol see section 5.3).

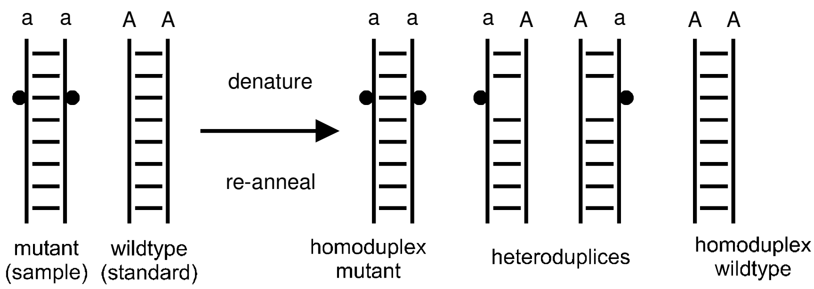


Figure 18: Principle of heteroduplex analysis.
The re-annealing of sample and standard results in 4 different DNA fragments. 1) The wildtype homoduplex (AA), 2) the mutant homoduplex (aa), 3) and 4) two different heteroduplices (Aa and aA). These heteroduplices carry at least one mismatch (disturbed base pairing) and have a significant lower melting temperature than the homoduplices.

This procedure results in a complete denaturing of both double stranded PCR fragments and a subsequent re-annealing. If the sample is different from the standard, re-annealing leads to 4 different double stranded DNA fragments (see Figure 18): 1) the homoduplex of the standard (wildtype AA), 2) the homoduplex of the sample (mutant aa),3) and 4) two heteroduplices between standard and sample (Aa and aA). Due to the differences between sample and standard these heteroduplices display mismatches in their base pairing in least one position. Such mismatches have a strong impact on the melting behavior because the

number of base pairs between the two strands is reduced. Therefore the heteroduplices can be easily separated from the homoduplices using TGGE.

The identification of the optimum temperature gradient for the separation of a heteroduplex analysis is absolutely the same as for a single fragment. The separation of a heteroduplex sample in a perpendicular TGGE results in 4 different melting curves. The 2 heteroduplices have a lower melting temperature and denature at a lower temperature compared to the homoduplices.

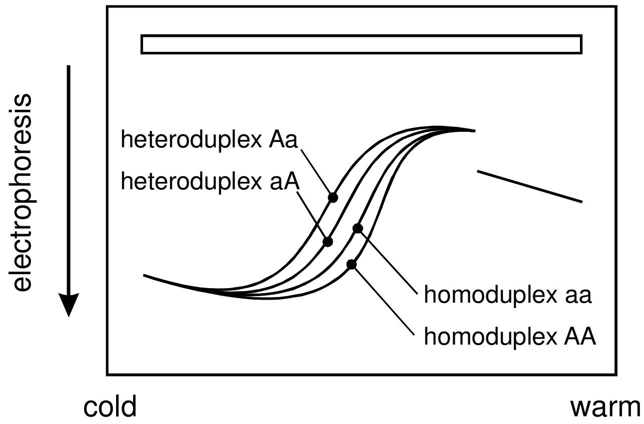


Figure 19: Separation of a heteroduplex sample in **perpendicular TGGE**.

The temperature gradient can then be adapted in the same way as for a conventional sample (see chapter 4.4). In parallel TGGE, the samples melt as they migrate along the temperature gradient. The heteroduplices (with mismatch) melt at a lower temperature than the homoduplices. Thus they open earlier in the partial single strand and are slowed down in the gel matrix. The homoduplices migrate a longer distance as complete double strands and start to melt at a higher temperature (i.e. later in respect to the temperature gradient). Therefore the lower bands in parallel TGGE are the homoduplices, whereas the higher bands are the heteroduplices.

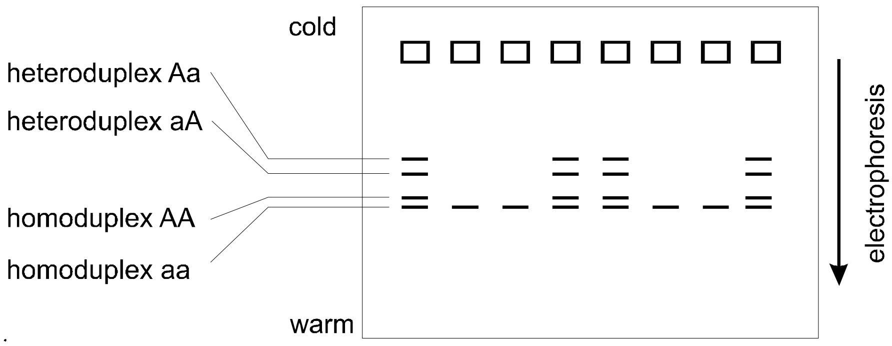


Figure 20: Schematic drawing of a screening multiple samples in a **parallel TGGE**. Both homoduplices (AA, aa) have a higher melting temperature and migrate further in the gel. The heteroduplices melt at a lower temperature resulting in a slower migration.

9.3.2 Evaluation of a heteroduplex analysis

There are two possible states in heteroduplex analysis: 1) the sample is identical to the standard (wildtype) 2) the sample is different from the wildtype. In the former case, the denaturation / renaturation procedure results in one (the same) homoduplex. The

subsequent separation in parallel TGGE shows only a single band. In the latter case, denaturation / renaturation leads to the four different populations depicted in Figure 19. Separation in parallel TGGE results in up to four different bands (see Figure 20). If the temperature gradient has not been correctly optimized, or if separation time was to short, there may as well only be two or three bands.

This makes heteroduplex analysis very easy to evaluate:

number of bands	**result**
one	sample is identical to the standard **no mutation**
more than one (up to 4 bands)	sample is different form the standard **mutation**

9.4 The TGGE Test kit

The TGGE test kit (024-050) was developed to get familiar with the TGGE system. It consists of 3 different DNA samples:

- a wild type sample (DNA fragment without mutation)
- a mutant sample (DNA fragment that differs in on position from the wild type)
- the heteroduplex sample (sample has been prepared as described in section 9.3.1)

The samples are separated in a **8% PAA** gel with **8M Urea** and a **1 x TAE** buffer system (for preparation of gel solution and buffer see section 17.2).

9.4.1 Perpendicular TGGE using the Biometra TGGE test kit

1) sample preparation

mix **100 µl** heteroduplex sample with
100 µl loading buffer TAE (see section 17.2)

2) Load heteroduplex sample to the broad slot of a perpendicular gel

3) Let sample migrate into the gel with 350V for approx. 20 minutes

4) Cover gel with cover film, assemble buffer wicks, cover plate and safety lid

5) start run

Temperature gradient	30 to 70°C
Voltage:	300V
Run time:	4h

6) silver stain gel

9.4.2 Parallel TGGE using the Biometra TGGE test kit

1) sample preparation:
mix **5µl sample** (wildtype or mutant or heteroduplex) with
10µl running buffer (TAE) and add
15µl loading buffer TAE (see section 17.2)

2) Assemble electrophoresis unit, cover gel with cover film (beneath the slots), assemble cover plate and safety lid

3) Load 5 µl of wildtype, mutant and heteroduplex samples

6) Let sample migrate into the gel with 400V for approx. 5 minutes

4) After pre-run, cover gel like described in section 6.2.2

5) start main run

Temperature gradient	30 to 60 °C
Voltage:	400V
Run time:	3 h

4) silver stain gel

10. Maintenance and repair

10.1 Cleaning and Maintenance

The TGGE system is built to operate for a long time without the need for periodical maintenance. Nevertheless, occasionally cleaning of the air inlet may be necessary to maintain the efficiency of the thermoblock. The inlet for the airflow is located at sides of the themoblock. Be sure that the inlet is not clogged by dust or other materials. Dust can be removed easily from the inlet with a conventional vacuum cleaner. Additionally, the housing may be cleaned from time to time with a smooth cotton cloth. Do not use strong detergents, abrasives or organic solvents for cleaning.

Important: Appropriate safety regulations must be observed when working with infectious or pathogenic material.

10.2 Servicing and repair

The TGGE system contains no user serviceable parts. Do not open the housing instrument. Service and repair may only be carried out by the Biometra Service department or otherwise qualified technical personal.

10.3 Replacement of Spare Parts

Only original spare parts mentioned in these operating instructions are allowed.

11. Accessories

11.1 Consumables and spare parts

TGGE MAXI Starter kit (Germany only) for 25 gels, including glass plate 1 slot (024-228), glass plate with spacer, no slot former (024-227), applicator strip silicone, 34 slots, 8µl (024-223), 2 cover plates (024-221), 2 sealings (024-230), 25 cover films (024-232), 25 Polybond films (024-234), 4 buffer wicks (024-216), sample AcrylGlide™ (211-319), 12 binder clamps (024-207)	024-204
TGGE MAXI Starter kit (International) for 25 gels, including glass plate 1 slot (024-228), glass plate with spacer, no slot former (024-227), applicator strip silicone, 34 slots, 8µl (024-223), 2 cover plates (024-221), 2 sealings (024-230), 25 cover films (024-232), 25 Polybond films (024-234), 4 buffer wicks (024-216),12 binder clamps (024-207)	024-294
TGGE Test kit Test DNA for perpendicular and parallel test runs: wild type DNA (control), mutant DNA, hetero duplex DNA, sample buffer.	024-050
TGGE MAXI buffer wicks, 100/pcs. (18 x 20 cm)	024-215
TGGE MAXI buffer wicks, reusable, 8 pcs.	024-217
TGGE MAXI glass plate without Spacer 23.5 x 23.5 cm	024-221
Self adhesive slot forming units (8 strips with 28 units á 5µl each for parallel gels, 9 strips with one broad slot á 200µl each for perpendicular gels)	024-222
Applicator strips 240mm (3 pcs.), 43 slots á 8µl each	024-223
TGGE Maxi glass plate with spacers and no slot fomers for use with applicator strips (024-223)	024-227
TGGE MAXI glass plate perpendicular, with spacer (1mm) and slot former (1 slot, 75 µl)	024-228
TGGE MAXI glass plate parallel, with spacer (1mm) and slot former (32 slots, 5 µl)	024-229
TGGE MAXI silicon sealing for casting of gels, 1mm	024-230
TGGE MAXI gel cover film 25/pkg	024-232
TGGE MAXI polybond film 25/pkg	024-234
TGGE MAXI polybond film 100/pkg	024-235

12. Service

Should you have any problems with this unit, please contact our service department or your local Biometra dealer:

Biometra GmbH
Service Department
Rudolf-Wissell-Straße 14 - 16
D-37079 Göttingen
Phone:++49 (0)5 51 50 68 6 - 10 or 12
Fax: ++49 (0)5 51 50 68 6 -11
e-mail: Service@biometra.com

If you would like to send the unit back to us, please read the following return instructions in chapter 12.1.

12.1 Instructions for return shipment

In case of an instrument failure that cannot be fixed by the procedures described in section 9 please proceed as follows:

- Return only defective devices. For technical problems which are not definitively recognisable as device faults please contact the Technical Service Department at Biometra (Tel.: +49 551-50881-10/12, Fax: +49 551-50881-11, e-mail: service@biometra.com).
- Please contact our service department for providing **a return authorization number (RAN).** This number has to be applied clearly visible to the outer box. **Returns without the RAN will not be accepted!**
- Important: Carefully clean all parts of the instrument of biologically dangerous, chemical or radioactive contaminants. If an instrument is contaminated, Biometra will be forced to refuse to accept the device. The sender of the repair order will be held liable for possible losses resulting from insufficient decontamination of the device.
- Please prepare written confirmation that the device is free from biologically dangerous and radioactive contaminants. The declaration of decontamination (see section 13) must be attached to the outside of the packaging.
- Use the original packing material. If not available, contact Biometra or your local distributor.
- Label the outside of the box with “CAUTION! SENSITIVE ELECTRONIC INSTRUMENT!”
- Please enclose a note which contains the following:
  a) Sender’s name and address,
  b) Name of a contact person for further inquiries with telephone number,
  c) Description of the fault, which also reveals during which procedures the fault occurred, if possible

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13. Equipment Decontamination Certificate

To enable us to comply with german law (i.e. §71 StrlSchV, §17 GefStoffV and §19 ChemG) and to avoid exposure to hazardous materials during handling or repair, please complete this form, prior to the equipment leaving your laboratory.

COMPANY / INSTITUTE ____

ADDRESS ____

PHONE NO ________________________ FAX NO ________________________

E-MAIL ____

EQUIPMENT Model Serial No

_______________ _______________

_______________ _______________

_______________ _______________

_______________ _______________

If on loan / evaluation Start Date: ________________ Finish Date ________________

Hazardous materials used with this equipment:

____

____

____

Method of cleaning / decontamination:

____

____

____

The equipment has been cleaned and decontaminated:

NAME ______________________________ POSITION ____________________
(HEAD OF DIV./ DEP./ INSTITUTE / COMPANY)

SIGNED ______________________________ DATE ________________________

PLEASE RETURN THIS FORM TO BIOMETRA GMBH OR YOUR LOCAL BIOMETRA DISTRIBUTOR TOGETHER WITH THE EQUIPMENT.
PLEASE ATTACH THIS CERTIFICATE OUTSIDE THE PACKAGING. INSTRUMENTS WITHOUT THIS CERTIFICATE ATTACHED WILL BE RETURNED TO SENDER.

General Information for Decontamination:

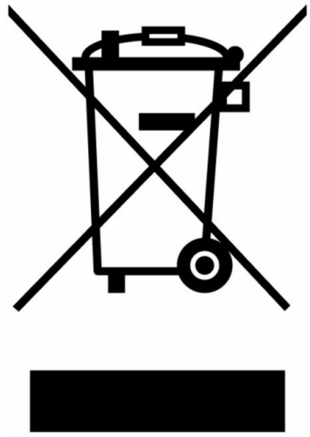
Please contact your responsible health & safety officer for details.

Use of radioactive substances:
Please contact your responsible person for details.

Use of genetically change organism or parts of those:
Please contact your responsible person for details.

14. Note for the disposal of electric / electronic waste.

Note	**for disposal of electric / electronic waste**
Hinweis	**für die Entsorgung von Elektroaltgeräten**
Renseignement	**du traitement des déchets des appareils électrique / électronique**



This symbol (the crossed-out wheelie bin) means, that this product should be brought to the return and / or separate systems available to end-users according to yours country regulations, when this product has reached the end of its lifetime.

For details, please contact your local distributor!

This symbol applies only to the countries within the EEA*.
EEA = European Economics Area, comprising all EU-members plus Norway, Iceland and Liechtenstein.

Dieses Symbol (die durchgestrichene Abfalltonne) bedeutet, dass dieses Produkt von der Firma Biometra für eine kostenlose Entsorgung zurückgenommen wird. Dies gilt nur für Geräte, die innerhalb Deutschlands gekauft worden sind.

Kontaktieren Sie für die Entsorgung bitte die Biometra Service-Abteilung!
Außerhalb Deutschlands wenden Sie sich bitte an den lokalen Händler.

Dieses Symbol gilt nur in Staaten des EWR*.
***EWR = Europäischer Wirtschaftsraum, umfasst die EU-Mitgliedsstaaten sowie Norwegen, Island und Liechtenstein.**

Cet symbol (conteneur à déchets barré d´une croix) signifie que le produit, en fin de vie, doit être retourné à un des systèmes de collecte mis à la disposition des utilisateurs finaux en conséquence des régulations par la loi de votre pays.
Pour des information additionel nous Vous demandons de contacter votre distributeur!

Cet symbole s´ápplique uniquement aux pays de l´EEE*.
EEE = Espace économique européen, qui regroupe les États membres de l´UE et la Norvège, Islande et le Liechtenstein.

15. Declaration of Conformity

EU – Konformitätserklärung
EC - Declaration of Conformity

Göttingen, 19. November 2008

im Sinne der EG-Richtlinie über elektrische Betriebsmittel zur Verwendung innerhalb bestimmter Spannungsgrenzen 73/23/EWG
following the EC directive about electrical equipment for use within certain limits of voltage 73/23/EEC

und / *and*

im Sinne der EG-Richtlinie für die elektromagnetische Verträglichkeit 89/336/EWG.
following the EC directive about the electromagnetic compatibility 89/336/EEC .

Hiermit erklären wir, dass folgende **Elektrophoresegeräte:**
*Herewith we declare that the following **gel electrophoresis systems:***

Typen / *types*:	TGGE MAXI System (German) TGGE MAXI System (International) TGGE MAXI Controller TGGE MAXI Elektrophorese Kammer mit Temperierblock TGGE MAXI Power Supply
	TGGE MAXI system (German) *TGGE MAXI system (International)* *TGGE MAXI controller* *TGGE MAXI electrophoresis unit with Peltier element powered gradient block* *TGGE MAXI power supply*
Best.-Nr. / *Order No.:*	024-200, 024-294, 024-201, 024-202, 024-203, 024-290

den grundlegenden Anforderungen der
corresponds to the basic requirements of

EG-Niederspannungsrichtlinie 73/23/EWG und der
EC low voltage directive 73/23/EEC and the

EG-Richtlinie über die elektromagnetische Verträglichkeit 89/336/EWG entsprechen.
EC directive about the electromagnetic compatibility 89/336/EEC .

Folgende harmonisierte Normen wurden angewandt:
The following harmonized standards have been used:

EN 55011 + A1 + A2	EN 55022 + A1 + A2
EN 61000-3-2	EN 61000-3-3 + A1
EN 61000-6-1	
EN 61010-1	EN 61010-2-010 + A1

Dr. Jürgen Otte
Quality Manager

Formular 006 Formular 006, Ver. 03

16. Warranty

This Biometra instrument has been carefully build, inspected and quality controlled before dispatch. Hereby Biometra warrants that this instrument conforms to the specifications given in this manual. This warranty covers defects in materials or workmanship as described under the following conditions:

This warranty is valid for 24 month from date of shipment to the customer from Biometra. This warranty will not be extended to a third party without a written agreement of Biometra.

This warranty covers only the instrument and all original accessories delivered with the instrument. This warranty is valid only if the instrument is operated as described in the manual.

Biometra will repair or replace each part which is returned and found to be defective. This warranty does not apply to wear from normal use, failure to follow operating instructions, negligence or to parts altered or abused.

17. Appendix

17.1 References

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17.2 Buffers

17.2.1 Running buffers:

TBE Running buffer 0.1 x conc. TBE (up to 1 x conc. TBE is possible)

10 x TBE (stock solution)	890 mM Boric Acid
	20 mM EDTA
	890 mM TRIS
	Do not titrate to adjust pH!

TAE Running Buffer 1 x conc. TAE, pH 8.0

50 x TAE (stock solution)	242g Tris base (2M)
pH 8.0	57,1 ml glacial acid
	100ml 0.5M EDTA (pH 8.0)

MOPS-Running Buffer 1 x conc. MOPS

50x MOPS (stock solution) **MOPS/EDTA** (ME) (50 x conc.)	1M MOPS
	50 mM EDTA
	pH = 8.0

17.2.2 Loading buffers:

Loading buffer TBE	TBE running buffer
	0.1% Triton-X 100
	0.01% Bromophenol Blue dye
	0.01% Xylene Cyanol dye

Loading buffer TAE	TAE running buffer
	0.1% Triton-X 100
	0.01% Bromophenol Blue dye
	0.01% Xylene Cyanol dye
	2 mM EDTA

Loading buffer MOPS	MOPS running buffer
	1 mM EDTA
	0.05% Bromophenol Blue dye
	0.05% Xylene Cyanol dye
	pH = 8.0

17.3 Other buffers:

TE buffer 10 mM Tris/HCl
0.1 mM EDTA
pH = 8.0

TEMED Solution of N,N,N',N'tetramethylethylendiamine

APS 10% Ammonium persulfate

Glycerol 40% 40% glycerol in water

Glycerol 50% 50% glycerol in water

18. Subject index